difference between these two worlds is now simply the sample format, i.e., cuvette vs. microplate. It seems reasonable to assume that the cuvette with its straightforward implementation of right angle excitation-emission geometry offers significant advantages over the epi-illumination geometry and uncontained sample imposed by a microplate when it comes to data quality. We have implemented a prototype microplate reader equipped with a variety of pulsed laser sources for measurement of fluorescence spectra, fluorescence lifetimes, and anisotropy. The subject of this poster is benchmarking its performance relative to cuvette format. The plate reader employs direct waveform recording as an alternative to TCSPC; studies to compare the speed, accuracy, and precision of the two lifetime approaches are presented along with several examples of titration curves for rapid determination of binding affinities via time-resolved FRET.

1772-Pos Board B664
Fluorescence Quenching of Tryptophan and Tryptophanyl Dipeptides in Solution
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We report measurements of fluorescence quantum yields of tryptophan, tryptophanylaspartate and tryptophanylarginine in several solvents as well as in aqueous solutions over a wide range of pH. We aim to test a computational model developed by Callis and coworkers [Vivian, J.T. and Callis, P.R. Chem. Phys. Lett. **2002**, *369*, 409] of fluorescence quantum yield, which postulates that quenching in tryptophan arises from energy loss due to an electron transfer from the aromatic system of tryptophan to one of the amides in the protein backbone. Since the electron transfer state is expected to be high in energy, normally this would not be a possible outcome, but because of its large dipole, such a state should be more accessible in polar solvents. In addition, conditions of low (high) pH, which result in a net positive (negative) charge for the backbone should result in an increase (decrease) of electron transfer rates and low (high) quantum yields. The observed results confirm the predictions of the model.

1773-Pos Board B665
Multiscale Diffusion of Single Molecules in Biomimetic Crowding
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Molecular crowding in living cells is believed to influence diffusion processes, intermolecular interactions, protein folding, and intracellular transport. Here, we have investigated crowding effects on the rotational and translation diffusion of Rhodamine green (RhG) and enhanced green fluorescent proteins (EGFP); as compared with homogeneous solvents (buffer and glycerol). Time-resolved fluorescence anisotropy (picoseconds - nanoseconds) and fluorescence correlation spectroscopy (microseconds - seconds) were used to elucidate the effect of non-specific binding on of RhG and EGFP diffusion in synthetic (Ficoll-70, Ficoll-400) and proteins (bovine serum albumin, BSA, and ovalbumin) biomimetic crowding. Using Stocks-Einstein model, the measured rotational-to-translational diffusion coefficient ratios of RhG and EGFP indicate that the non-specific binding and deviation from Brownian diffusion depend on the type of crowding agents. These results provide new insights into crowding effects on diffusion and nonspecific binding of fluorophores on multiple scales of time and concentration.

1774-Pos Board B666
Characterization of Fluorescent Base Analogs to Study DNA Base Flipping at the Single Molecule Level
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Chemical damage to DNA bases can result in mutations, block replication, and lead to cancer. It has been suggested that the phenomenon of base flipping take place by some enzymes during the repair of DNA damages. However, it still remains to be answered if the enzyme ''pushes'' the nucleotide out of the helix (active mechanism) or if the enzyme binds to a provisional flipped base (passive mechanism). Single molecule fluorescence has demonstrated to be a powerful technique to determine the formation of one or more intermediates, and to study the kinetics of the processes from the instant before an enzyme interact with the DNA until the release of the enzymatic product, one molecule at a time. Therefore, in order to optimize and maximize the repair of damaged DNA, new single molecule approaches to fully assess the kinetic mechanism of the base flipping process are needed.
In previous work, the adenine fluorescent base analog 2-aminopurine (2AP) has been extensively used to study base flipping in ensemble average experiments. In addition, a novel 2AP single molecule approach was recently developed.[1] In order to generate single molecule fluorescence assays to probe base flipping in different DNA-enzyme complexes, we need to study fluorescent base analogs (FBA) for all the natural bases. Several FBA molecules have been synthesized during the last four decades and we have selected one FBA molecule for each DNA base to probe base flipping. We have characterized the fluorescent properties of different FBA-substituted DNA molecules that mimic the different states proposed for the base flipping process.
[1]Alemán, E.A., Patrick, E., de Silva, C., Musier-Forsyth, K. & Rueda, D. Single-molecule dynamics with fluorescent nucleotide analogues. *In preparationto be submitted*

1775-Pos Board B667
Temporal Dynamics and FRET Restrained Modeling of an "Invisible" Excited State of T4 Lysozyme
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Conformational fluctuations play a central role in enzyme catalysis. However, extracting a 4D view, i.e. structural changes over time, has represented a big challenge in molecular biophysics. For example, in most cases not all conformers of proteins are visible using the standard structural techniques such as X-ray crystallography or nuclear magnetic resonance. In this work, we present our approach using a fluorescence spectroscopic toolbox to resolve three different conformers of the bacteriophage T4 lysozyme (T4L) and their dynamics. We created a set of more than 20 double mutants specifically labeled with a Förster resonance energy transfer (FRET) pair via the insertion of an unnatural amino acid and a single cysteine. Ensemble time correlated single photon counting (eTCSPC) revealed their corresponding population fractions and provides with structural information. Nevertheless, single molecule FRET, in confocal illumination, showed fluorescence lifetime averaging in timescales faster than diffusion time. To fully characterize the dynamics we used filtered fluorescence correlation spectroscopy which combined with eTCSPC represent a time resolution of seven orders of magnitude (ns to ms). In all, we used the measured distance network to generate a FRET restrained model of the three conformers with high precision. The open conformer appears readily available for substrate binding; the close conformer is very similar to the covalent enzyme-substrate adduct in the T26E mutant of T4L; the third conformer appears more compact than the adduct form which, at present, has not been reported in over more than 440 entries in the protein data bank.

1776-Pos Board B668
Photoblinking and Photobleaching of Single Molecule Fluorescent Probes Induced by Mn^{2+}
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Photophysical phenomena leading to blinking and irreversible photobleaching represent a major obstacle that limits the utility of organic fluorophores in fluorescence-based techniques involving the detection of small numbers of molecules. In recent work, we demonstrated that the coordination of paramagnetic transition metals (e.g. Mn^{2+}) to DNA induces intersystem-crossing in dyes covalently attached to the nucleic acid. This results in fluorescence quenching, triplet blinking and accelerated photobleaching. The increase in triplet formation in the presence of manganese was demonstrated using transient absorption techniques and fluorescence correlation spectroscopy for a series of rhodamine and cyanine dyes, including TAMRA, Cy3 and Cy5. These results are particularly relevant for single-molecule or fluorescence correlation spectroscopy experiments aimed to study enzymes that act on DNA, where Mn^{2+} is used to relax the sequence-specificity of enzymes that catalyze phosphoryl transfer reactions (e.g. polymerases and restriction endonucleases).

1777-Pos Board B669
One and Two Photon Fluorescence Correlation Spectroscopy on Proteins in Glucose Solutions
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Cryopreservation is a powerful technology with many applications in biomedical fields from organ preservation to cellular research. Simple sugars such as glucose and fructose are among the most widely used cryopreserving agents, yet despite their widespread use, the mechanism through which sugars protect